

A² administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising [at least one of the following nucleic acid sequences]

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

Please add the following new claims.

36. (New) The cell of claim 5 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the lethal gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

37. (New) The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

38. (New) The cell of claim 17 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the permissive environment.